Lactose, melibiose and glycerol, however, were not fermented but oxidized, that is, acid was produced from these sugars within 48 hr. under aerobic conditions only.

In shaken, aerobic cultures at 30° C. of strain *B1x*, containing 2 per cent (w/v) of lactose or melibiose, all reducing power disappeared within 48 hr. Paper chromatograms of culture filtrates, using *n*-propanol/ammonia (70 : 30) as solvent and sprayed with ammoniacal silver nitrate or thymol blue, showed respectively spots corresponding to lactobionic acid and melibionic acid (prepared by the method of Levene and Jorpes[2]), which were absent from control cultures without sugar. Comparison of the sizes of the spots with those of known concentrations of the acids suggested that the conversion of sugar to acid had, in each case, been nearly quantitative.

Confirmatory evidence of the identity of the acids was obtained by subjecting the filtrates, after passage over 'Zeo-Karb 225', to hydrolysis with *N* hydrochloric acid at 100° C. for 2 hr., removing the acid with silver oxide and running the resultant solutions on paper using the same solvent. On spraying with ammoniacal silver nitrate, spots corresponding to galactose and gluconic acid, absent from the original filtrates, were obtained. The acids present in the original culture filtrates have therefore been provisionally identified as lactobionic and melibionic.

It seems that this paracolon strain is able to oxidize lactose and melibiose to lactobionic and melibionic acids respectively. The oxidation of lactose by paracolon bacteria[1], and the oxidation of lactose and maltose to the corresponding acids by oxidative *Pseudomonas*[3,4] and *Achromobacter*[5] spp. have been reported, but so far as is known, this is the first recorded instance of the production of melibionic acid from melibiose. The wider use of the Hugh and Leifson technique would probably reveal more cases of this kind. This work emphasizes the inadequacy of the traditional fermentation tube technique in which both aerobic and anaerobic conditions exist.

I wish to thank Dr. F. A. Isherwood and Mr. F. C. Barrett for their help and advice.

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Role of Oxygen in the Formation of α-Amino-butyric Acid by a Staphylococcus

THE formation of α-amino-butyric acid by animal cells has been studied[1,2], but little is known of the method of its formation by staphylococci[3].

It has been shown here, using shaken flasks, that the formation of α-amino-butyric acid by staphylococci is a function of the amount of oxygen available to the culture. It has been confirmed that the acid can be derived from threonine and it has been shown that, presumably by a similar process, alanine can be formed from serine.

The organism used was the *V*8 strain of staphylococcus employed by Gladstone and van Heyningen[4]. The culture medium, consisting of 'Bacto Casamino-acids', 2 per cent; yeast diffusate, 20 per cent; sodium lactate, 0·75 per cent; sodium glycerophosphate, 2 per cent; and mineral salts was the same as that used by Gladstone and van Heyningen. The organisms were grown at 37° C. in 500-ml. conical flasks plugged with cotton wool, the oxygen solution-rate being varied by varying the volume of medium in the flask. When shaken on the bottom shelf of a Kantorowicz[5] shaker the flask containing 1/5 its volume of liquid gave an oxygen solution-rate determined by sulphite oxidation[6] of 29 m.moles O_2/l./hr. and the flask containing 1/20 its volume of liquid gave a value of 75 m.moles O_2/l./hr.

Changes in amino-acid composition of the medium during growth were followed by two-way chromatography.

While no new amino-acids appeared during growth in the flasks containing 1/20 their volume of medium, a fresh acid appeared early in the logarithmic growth-phase in the flasks containing 1/5 their volume of medium. This was shown to be α-amino-butyric acid.

Cells from flasks with the lower oxygen solution-rate incubated with threonine formed α-amino-butyric acid; with serine, alanine was formed, while with aspartic acid, glutamic acid or methionine, no new material appeared. Cells from flasks with the higher oxygen solution-rate similarly treated produced no changes.

When nitrogen was substituted for air in sealed flasks with 1/20 vol. medium, α-amino-butyric acid was formed during growth. When both sodium lactate and sodium glycerophosphate were omitted from the medium, the staphylococci still grew well. It was shown that growth in such a medium in the flasks containing 1/5 vol. medium failed to produce α-amino-butyric acid, whereas with lactate and glycerophosphate present it was formed. Replacement of air by nitrogen when lactate and glycerophosphate were absent from the flasks containing 1/5 vol. medium brought about formation of α-amino-butyric acid.

When glucose or glycerol was substituted for lactate and glycerophosphate on an equimolar basis, α-amino-butyric acid was still formed during growth in flasks containing 1/5 vol. medium. The use of pyruvic acid, malic acid, formic acid, succinic acid or α-keto-glutaric acid as a substitute for lactate and glycerophosphate failed to bring about α-amino-butyric acid formation. Addition of pyruvic acid to the lactate-containing medium in the flasks with the lower oxygen solution-rates suppressed the formation of α-amino-butyric acid.

It would seem from these results that the formation of α-amino-butyric acid by this staphylococcus is due to the use of threonine as a hydrogen acceptor under conditions of low oxygen supply, serine presumably functioning in a similar manner.

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Jan. 9.

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